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Fusarium Toxins in Wheat Harvested During Six Years in an Area of Southwest Germany

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ABSTRACT A total of 84, 78, 80, 80, 78, and 45 wheat samples for feed use were collected randomly after the 1987, 1989, 1990, 1991, 1992, and 1993 crops, respectively, from farms in an area of southwest Germany. The sum of precipitation from May to September varied during years, with markedly higher precipitation in 1987, compared to 1989–1993. Deoxynivalenol, 3- and 15-acetyldeoxynivalenol, nivalenol, HT-2 toxin, T-2 toxin, diacetoxyscirpenol, and fusarenon-X were determined by gas chromatography, combined with mass selective detection (GC-MS), zearalenone, α- and β-zearalenol by GC-MS or HPLC. Deoxynivalenol was the major toxin, with incidences of 68–95% and mean contents at 152–1,692 μg/kg. In contrast, incidences of zearalenone, 3-acetyldeoxynivalenol, nivalenol, HT-2 toxin, and T-2 toxin were at 11–80, 17–60, 25–64, 0–8, and 0–41%, respectively, with mean contents between 3 and 209 μg/kg. α-zearalenol and/or β-zearalenol were detected in five samples at contents ≤71 μg/kg; diacetoxyscirpenol was not detected in any sample. 15-acetyldeoxynivalenol and fusarenon-X were assayed in samples from 1987 and 1991–1993. 15-acetyldeoxynivalenol was detected in 3–15% of samples at mean contents of 5–84 μg/kg; fusarenon-X was not detected. Over the years, incidences and levels of toxins remained constant, decreased or increased. The correlation between the occurrence of toxins and precipitation is discussed. *Nat. Toxins 5:24–30, 1997.*

Key Words: zearalenone; zearalenol; deoxynivalenol; acetyldeoxynivalenol; nivalenol; fusarenon-X; HT-2 toxin; T-2 toxin; diacetoxyscirpenol; wheat

INTRODUCTION

The occurrence of the *Fusarium* toxins, deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), nivalenol (NIV), HT-2 toxin (HT-2), T-2 toxin (T-2), diacetoxyscirpenol (DAS), zearalenone (ZEA), and α- and β-zearalenol (α,β-ZOL) in wheat for feed use grown in 1987 in an area of southwest Germany has been described previously [Müller and Schwadorf, 1993]. This study was conducted because reliable data of the occurrence of *Fusarium* toxins in the area surveyed were not available. Monitoring was continued in wheat samples collected in the same area after harvest in 1989–1993. In samples from four years (1987, 1991–1993) wheat samples were assayed also for 15-acetyldeoxynivalenol (15-ADON) and fusarenon-X (FUS-X). Total precipitation in the summer months 1989–1993 was significantly below that measured in the summer 1987 and varied also during 1989–1993. It is well known that the development of fusaria and other pathogenic fungi in cereals in crops is strongly dependent on weather conditions [Snijders, 1990; Miller, 1994; Parry et al., 1995]. An effect of climate and in particular of precipitation on the occurrence of *Fusarium* toxins in cereals is indicated by data described by a variety of authors [Blaney et al., 1984, 1987; Hagler et al., 1987; Hussein et al., 1989; Langseth et al., 1995; Lauren et al., 1996]. Therefore, it was expected that the different climatic conditions during the years would be associated with differences in the pre-harvest occurrence of *Fusarium* toxins also in southwestern Germany.

This present study compares the data of all years surveyed. An investigation of the occurrence of *Fusarium* toxins in cereal samples collected randomly after harvest from an area in Europe during a period of six years has not yet been described in literature.

MATERIALS AND METHODS

Samples

A total of 84, 78, 80, 80, 78, and 45 wheat samples, grown in 1987, 1989, 1990, 1991, 1992, and 1993, respectively, were randomly selected 1–4 weeks following harvest from farms located in the Stuttgart governmental district, a northeastern part of the province of Baden-Wuerttemberg. Sampling was conducted by the Governmental Advisory Board. Samples were combine harvested and dried immediately or soon after the 1987 harvest but were mostly left undried after the 1989, 1990, 1991, 1992, and 1993 harvests. Samples (700–1,000g) were stored at −18°C prior to examination of mycotoxins.

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Determination of Mycotoxins

Trichothecenes were extracted from finely ground samples (particle size about 0.3 mm) as described by Tanaka et al. [1985]. After trifluoracetylation, quantification and identification was done by capillary gas chromatography with selective mass detection as described by Schwadorf and Müller [1991]. ZEA, α-ZOL, and β-ZOL were extracted from the ground samples of the 1987 crop with ethylacetate; the ethylacetate extract was subjected to a base treatment and partitioned with water; toxins were identified and quantified as the trimethylsilyl derivatives by capillary gas chromatography with selective mass detection as described by Schwadorf and Müller [1992]. ZEA, α-ZOL and β-ZOL were extracted from the 1989, 1990, 1991, 1992, and 1993 crop samples according to Tanaka et al. [1985] and quantified by HPLC with fluorescence detection (ZEA, α-ZOL) or UV-detection (β-ZOL). The HPLC equipment consisted of an isocratic HPLC pump (ConstaMetric III metering pump, TSP, Darmstadt, Germany), a programmable fluorescence detector (HP 1046A, Hewlett Packard, Waldbronn, Germany), a UV-detector (TMD variable wavelength detector, TSP, Darmstadt, Germany), an injection valve with 20 and 100 µl loops (Model 7125; Rheodyne Inc., Cotati, CA), injection syringes 25 and 100 µl (Hamilton, Bonaduz, Switzerland), a LC column LiChrosorb Si 60, 5 µm, 250 × 4.6 mm (Merck, Darmstadt, Germany), an integrator (Cl-10B) and plotter (both TSP), and a floppy disk drive (2031 LP, Commodore, Germany). As eluent dichloromethane (saturated with water)/1-propanol (98.5/1.5, v/v) was used, flow rate was adjusted to 1.8 ml/min, fluorescence detection was at 235 nm (excitation) and 450 nm (emission), UV-detection at 236 nm, integration was from valley to valley, using an external standard for calibration. Detection limits of GC-MS were 1–2 µg/kg (ZEA, α- and β-ZOL) and 1–5 µg/kg (DON, 3-ADON, 15-ADON, FUS-X, NIV, T-2, HT-2, DAS), detection limits of HPLC were 0.5 µg/kg (ZEA), 1 µg/kg (α-ZOL) and 5 µg/kg (β-ZOL).

Moisture Content

The moisture content of blended wheat samples was determined by drying at 105°C for 4 h.

Precipitation Data

These data were measured at 25 weather stations located through the whole area of the Stuttgart governmental district.

Statistics

Normal distribution of toxin contents was checked by using the procedure 'proc univariate normal' of the SAS/STAT software (SAS Institute, Inc., 1987, Release 6.04). Toxin contents and weather data were not normally distributed in all cases. Therefore, a two sample t-test applied to the ranks was used for examination of statistical significance of differences. It is equivalent to a Wilcoxon rank sum test using the t-approximation for the significant level and was performed by the procedure 'proc npar1way wilcoxon' of the SAS.

RESULTS

Precipitation and Moisture Content of Samples

The total sum of precipitation from May to September measured during six years at 25 weather stations located in the surveyed area is summarized in Table I. During these months significantly more precipitation occurred in 1987 compared to 1989–1993. Precipitation was lowest in 1989 and 1991. During these years it was 1.9 times lower than 1987. It increased in 1990, 1992, and 1993 reaching a maximum in 1993. However, even during these years it was significantly below that in 1987.

The moisture content of samples at the time of sampling ranged between 9.5 and 17.9% with mean values between 12.3 and 14.7% (Table I). In 1987 these low moisture contents resulted mostly from high temperature drying of wheat immediately after harvest, in the other years preservation mostly was not necessary because of dry weather conditions.

Ranking of *Fusarium* Toxins

The occurrence of *Fusarium* toxins in wheat is summarized in Table II. ZEA, α- and β-ZOL, DON, 3-ADON, NIV, HT-2, T-2, and DAS were analysed in samples from six years, 15-ADON and FUS-X in those from four years. Mean levels were calculated from the concentration of toxins found in positive samples.

Based on incidence, mean and maximum contents, DON was the major toxin. The percentage of samples positive for this toxin ranged between 69 and 96% whereas the frequency of the other toxins did not exceed 64%, except for the 80% incidence of ZEA in samples harvested in 1987. The difference between DON and the other toxins is even more pronounced if mean and maximum levels of concentration are compared. Mean DON contents ranged between 152 and 1,692 µg/kg, maximum levels from 1,187 to 20,538 µg/kg. However, mean and maximum contents of the other toxins did not exceed 84 and 249 µg/kg, respectively, except for the higher levels of ZEA and 3-ADON in samples from 1987 and 1993, respectively. It is noteworthy that these levels were lower than the corresponding DON contents. Incidences and levels of 15-ADON were similar to or lower than the corresponding values of 3-ADON. α-ZOL was found in four samples from 1987 and in one sample from 1993, β-ZOL in one sample from 1987, at contents ≤71 µg/kg. DAS and FUS-X were not detected in any sample.

Differences Between Years

With regard to the incidence of DON there was a considerable consistency in the data from five years. Detectable amounts of this toxin were found in 95–96% of samples collected in 1987 and in 1990–1993 and it was only in

TABLE I. Total Precipitation From May to September Measured at 25 Different Stations Within the Stuttgart Governmental District (Province of Baden-Wuerttemberg, Germany) and Moisture Content of Wheat Samples Harvested in This Area

Crop year	Total precipitation (mm)		Moisture content (%)	
	Range	Mean*	Range	Mean
1987	335–844	548 ± 118[a]	11.3–17.9	14.7 ± 1.3
1989	162–380	282 ± 53[b]	11.3–17.5	14.4 ± 1.3
1990	246–467	327 ± 54[c]	9.8–15.6	12.3 ± 1.5
1991	154–446	289 ± 76[b]	9.5–14.5	12.4 ± 1.0
1992	296–524	381 ± 62[d]	10.3–15.4	12.4 ± 1.1
1993	226–619	409 ± 105[d]	12.5–16.7	14.7 ± 1.1

*Values within a column with different superscripts differ at $P < 0.05$.

samples from 1989 that DON was found at lower frequency (69%). In contrast, differences between DON levels of positive samples were much higher. As can be seen from Table II markedly higher mean and maximum contents were found in samples collected in 1987 compared to those from 1989–1993. In these latter years mean and maximum contents ranged from 9 to 35% and from 6 to 44%, respectively, expressed in percent of the 1987 contents.

The most striking difference between years was the markedly lower occurrence of ZEA in samples from 1989 to 1992 compared to those from 1987 and 1993 (Table II). In 1989–1992 the percentage of samples with detectable ZEA expressed as percent of positive samples detected in 1987 ranged between 14 and 24%. Likewise, mean contents of positive samples and maximum contents ranged at 0.2–11.4% and at 0.1–1.4%, respectively, expressed as percent of the corresponding levels in samples from 1987. In 1993, the frequency of ZEA increased to 62%, whereas its mean and maximum contents were similar to those determined in samples from 1989–1992.

Incidences and levels of 3-ADON, 15-ADON and NIV decreased or increased over the years (Table II). The percentage of samples with detectable 3-ADON decreased continuously from 60% in 1987 to 17% in 1992. In contrast, the frequency of NIV increased from 25% in 1987 to 64% in 1992. From 1992 to 1993 the frequency of 3-ADON increased whereas that of NIV decreased. Somewhat different changes were found with toxin levels. Mean and maximum contents of 3-ADON increased during the first years, then decreased but increased again. Mean and maximum NIV contents also increased during the first years and then decreased. 15-ADON behaved similar to 3-ADON. The frequencies and levels of HT-2 toxin and T-2 toxin also showed variable responses over the years (Table II).

Differences between mean contents of different years were only partly significant (Table II). Insignificant differences may result from high coefficients of variation, e.g., of mean DON and ZEA contents, from low numbers of positive samples and/or from small differences between years. High coefficients of variation often are associated with the ab-

TABLE II. *Fusarium* Toxins in Wheat Samples Harvested During Six Years in the Stuttgart Governmental District (Province of Baden-Wuerttemberg, Germany)

Toxin	Harvest	Samples positive (%)[f]	Toxin in positive samples (µg/kg)	
			Range	Mean*
ZEA	1987	80	1–8036	178.0 ± 994.3[a]
	1989	14	1–6	3.2 ± 1.7[bc]
	1990	11	1–15	5.1 ± 4.4[ac]
	1991	13	1–109	20.3 ± 32.5[a]
	1992	19	1–20	4.3 ± 5.1[c]
	1993	62	2–52	11.1 ± 13.0[a]
α-ZOL	1987	5	4–71	22.6 ± 32.2
	1989–1992	0		
	1993	2	8	8.4
β-ZOL	1987	1	12	12.0
	1989–1993	0		
DON	1987	96	4–20538	1691.6 ± 3993.1[ac]
	1989	69	3–1187	152.1 ± 213.7[b]
	1990	96	8–8969	595.0 ± 1221.8[c]
	1991	96	4–4627	359.1 ± 740.7[ab]
	1992	95	18–5412	334.8 ± 663.5[a]
	1993	96	19–6165	391.2 ± 1001.6[a]
3-ADON	1987	60	3–18	6.7 ± 3.6[a]
	1989	31	3–12	7.0 ± 2.8[a]
	1990	25	2–25	11.9 ± 6.4[b]
	1991	21	13–120	49.5 ± 30.6[c]
	1992	17	10–44	20.2 ± 10.5[d]
	1993	62	10–1902	209.2 ± 373.9[e]
15-ADON[g]	1987	15	1–15	5.0 ± 4.2[a]
	1991	13	5–150	41.9 ± 44.9[b]
	1992	3	6–116	61.0 ± 77.8[ab]
	1993	7	7–181	71.0 ± 95.7[b]
NIV	1987	25	3–32	9.0 ± 8.3[a]
	1989	42	3–58	19.5 ± 15.2[bc]
	1990	39	4–218	42.8 ± 54.2[bd]
	1991	59	1–188	22.1 ± 34.6[ac]
	1992	64	3–219	33.3 ± 38.6[d]
	1993	33	5–47	15.7 ± 14.6[c]
FUS-X[g]	1987	0		
	1991–1993	0		
HT-2	1987	7	2–20	8.8 ± 7.0[a]
	1989	8	12–22	17.2 ± 4.2[a]
	1990	1	17	17.0
	1991	0		
	1992	6	8–150	50.8 ± 59.0[a]
	1993	0		
T-2	1987	26	3–249	82.5 ± 78.3[a]
	1989	6	10–12	11.0 ± 1.0[b]
	1990	11	10–136	51.7 ± 48.7[a]
	1991	4	4–16	10.0 ± 6.0[ab]
	1992	0		
	1993	40	3–94	20.1 ± 23.1[b]
DAS	1987–1993	0		

*Values within a column with different superscripts (a–e) differ at $P < 0.05$ between years.

[f]Based on 84, 78, 80, 80, 78, and 45 samples collected in 1987, 1989, 1990, 1991, 1992, and 1993, respectively.

[g]15-ADON and FUS-X were not analysed in samples from 1989 and 1990.

sence of normal distribution of toxin contents. For example, DON contents in samples from 1987 and 1989 were not normally distributed, with coefficients of variation of 236 and 140%, respectively. Likewise, the coefficient of variation of atypically distributed ZEA content in samples from 1987 was 558%. These high coefficients of variation result from frequency distributions which are characterized by long tails towards high values.

Frequency Distribution of DON and ZEA and Risk for Livestock

In the Federal Republic of Germany, maximum limits for *Fusarium* toxins in grain have not yet been suggested. In Canada, maximum guidelines for levels of both ZEA and DON in grains used as animal feed have been suggested. According to Stratton et al. [1993], these guidelines are 5 mg DON/kg in grain for ruminants and poultry, 1 mg DON/kg in grain for swine, and 0.3 mg ZEA/kg in grain used as feed for ruminants, poultry and swine, with a zero tolerance for ZEA for lactating and pregnant animals.

As can be seen from Table III the percentage of samples with DON at levels above 5,000 µg/kg was at 9% in 1987, and between 0 and 3% in the other years. Likewise, the percentage of samples with DON exceeding 1,000 µg/kg was higher in 1987 compared to the other years. It was at 25% in 1987, whereas it ranged at 1–16% in the other years. Therefore, based on the Canadian limits the risks posed by DON in wheat for livestock seem to be generally low in the area surveyed, except for the risk for swine in a year with high precipitation.

The concentration of ZEA exceeded 300 µg/kg in 5% of the samples from 1987, but was below this limit in all other samples. Thus, based on the Canadian limits the risk for livestock was low in 1987 and did not exist during the other years. However, the risk posed by ZEA for lactating and pregnant sows was higher. Its concentration exceeded the detection limit in 80 and 62% of samples from 1987 and 1993, and in 11–19% of samples from the other years (Table II).

TABLE III. Frequency Distribution of DON in Wheat Samples Harvested During Six Years in the Stuttgart Governmental District (Province of Baden-Wuerttemberg, Germany)

	Percentage of samples with indicated toxin level					
Toxin level (µg/kg)	1987	1989	1990	1991	1992	1993
<100	42	69	26	54	45	51
100–500	19	27	43	32	37	36
>500–1,000	14	3	15	5	16	7
>1,000–2,000	12	1	12	4	1	2
>2,000–5,000	4	0	1	5	0	2
>5,000	9	0	3	0	1	2

DISCUSSION

Fusaria can grow and produce toxins in cereals not only in the field but also after harvest if the moisture content of kernels is high enough. Thus, DON and ZEA were formed to a considerable extent during ambient air drying of wheat [Langseth et al., 1993], and the production of DON, 3-ADON, and ZEA was observed during malting of barley [Schwarz et al., 1995]. The growth of fusaria in cereals requires a minimum moisture content at 20–22% [Christensen and Sauer, 1982; Abramson, 1991]. The moisture content of samples at the time of sampling was below this limit (Table I). This indicates that the growth and toxin formation of fusaria occurred exclusively in the field in each of the six years surveyed.

Numerous investigations on the natural occurrence of *Fusarium* toxins in wheat have been carried out during the last two decades in different parts of the world [Gareis et al., 1989; Scott, 1989; Sundheim et al., 1988; Tanaka et al., 1988, 1990; Tutelyan et al., 1990; Luo et al., 1990; Perkowski et al., 1990; Hietaniemi and Kumpulainen, 1991; Lauren et al., 1991; Stratton et al., 1993; Trucksess et al., 1995; Yoshizawa and Jin, 1995; Quiroga et al., 1995; Trigo-Stockli et al., 1995]. Generally, DON and ZEA were determined, whereas NIV, 3-ADON, 15-ADON, T-2, HT-2, DAS, and FUS-X were analysed less frequently. Only a few reports have been published also on the occurrence of α- and β-ZOL [Müller and Schwadorf, 1993].

Incidence data and toxin levels described in the literature cannot be compared closely to that of the present study because of several drawbacks. The major one is the heterogeneity of samples examined by different authors. Samples were either randomly collected or selected on the basis of visible moulding or from clinical cases of intoxication. Furthermore, different methods were used for the determination of *Fusarium* toxins including gas chromatography combined with mass spectrometry, gas chromatography with flame ion ionisation detection (GC-FID) or electron capture detection (GC-ECD), high performance liquid chromatography, thin layer chromatography, differential pulse polarography, and enzyme immuno assays. Detection limits vary between these methods, and GC-FID and GC-ECD may cause false positive or negative results [Gareis et al., 1989]. In each of the six years of the present study, samples were randomly collected after harvest from farms located in the same area. Gas chromatography with selective mass detection, which is a sensitive and reliable method for the determination of trichothecenes, was used throughout.

In spite of these differences there are similarities between our results and those described in the literature.

In the present study DON was the predominating toxin, based on incidence and levels. This is consistent with other results. In a review of the occurrence of *Fusarium* toxins in cereals originating from European countries including the Federal Republic of Germany, Gareis et al. [1989] calculated the overall incidence of DON, NIV, 3-ADON, HT-2, T-2,

DAS, ZEA in wheat at 61, 39, 4, 5, 2, 1, 26%, respectively. Mean concentrations of DON were generally higher than those of NIV, ZEA and (with some exceptions) type A-trichothecenes. The corresponding overall frequencies in the present study were 91, 44, 34, 4, 13, 0, and 31%. Thus the ranking of toxins in these studies was principally the same as in the present investigation. The higher frequency of DON in our study may have resulted from the low detection limit of the method employed.

The predominating role of DON in wheat of European origin has been described also in recent publications. Among 13 Dutch wheat samples 100, 92, and 54% were contaminated with DON, NIV, and ZEA, respectively, with average concentrations at 115, 38, and 45 µg/kg [Tanaka et al., 1990]. In 47 Finnish wheat samples for food or feed use DON, 3-ADON, and ZEA were found at an incidence of 94, 28, and 8.5% and a mean concentration of 95, 39, and 30 µg/kg, respectively, while NIV, T-2, and HT-2 were not detected in any sample [Hietaniemi and Kumpulainen, 1991]. Perkowski et al. [1990] found DON but not NIV, 3-ADON and ZEA in three Polish wheat samples containing healthy looking kernels. Sundheim et al. [1988] analysed 40 wheat samples from South-Eastern and Central Norway and found DON, NIV, and ZEA at an incidence of 60, 100, and 7%, respectively, whereas T-2 and FUS-X were not detected. This is the only example known to us of a higher incidence of NIV compared to that of DON in wheat of European origin. However, even in this study the mean content of DON was higher than that of NIV. Tutelyan et al. [1990] analysed 14, 90, and 120 wheat samples harvested in the USSR in 1986, 1987 and 1988 for DON and found this toxin in 100, 62, and 93% of samples, respectively, at concentrations between 0.1 and 13.9 mg/kg. This contamination is similar to that found in our study (Table II).

In countries outside Europe other *Fusarium* toxins instead of DON may dominate [Scott, 1989]. Thus, in samples from Korea, Japan, and Nepal, NIV was found at higher frequencies and mean levels than DON and ZEA, and in some Korean samples even ZEA was detected at higher levels than DON [Tanaka et al., 1988]. However, in wheat samples from countries, such as Argentina, Canada, Taiwan, the incidence and mean concentration of DON was highest [Tanaka et al., 1988]. Results of Yoshizawa and Jin [1995] suggest regional differences in the DON and NIV contamination of wheat in Japan: DON was the major trichothecene in the northern districts and NIV in the central districts, whereas in the southern districts the DON level was similar or slightly higher than the NIV level. Luo et al. [1990] who analysed 30 wheat samples from two Chinese provinces for DON, NIV and ZEA confirmed the major role of DON. Quiroga et al. [1995] analysed a total of 1056 wheat samples harvested in various provinces of Argentina over a six year period (1985–1992). The incidence of DON, 3-ADON, and ZEA was at 22–71%, 0–5.4%, and 0–25%, respectively. The average concentration over positive samples of DON was markedly higher than that of ZEA, and with the exception of one year it was also higher than that of 3-ADON. Samples from 1986 were analysed for type A-trichothecenes (T-2, HT-2, DAS, neosolaniol). The incidence of these toxins was at 3–10%, their average content except that of DAS was markedly below that of DON.

It is important to note that DON in the present study was found at higher incidences and levels compared also to 3-ADON, 15-ADON, α- and β-ZOL. These toxins have been only rarely analysed so far in wheat and other cereals of European origin. In the southwestern part of Germany, α- and β-ZOL as well as DAS and FUS-X seem not to be of relevance in wheat.

A possible explanation of the dominance of DON may be that climatic conditions or other factors favoured the development of DON producing *Fusarium* strains and/or the formation of DON. In southern Germany, *F. graminearum* and *F. culmorum* are probable producers of DON [for literature see Müller and Schwadorf, 1993]. Two main chemotypes of *F. graminearum/G. zeae* have been described which produce either DON and ADON (3- /or 15-ADON) or NIV and FUS-X, with ZEA as a possible metabolite of both chemotypes [for literature see Müller and Schwadorf, 1993]. The predominance of DON in the present study thus may have resulted at least partly from the predominance of the DON-chemotype of *F. graminearum/G. zeae* in the fusaria present. This hypothesis is consistent with the complete absence of detectable FUS-X.

A further explanation of the dominance of DON may be due to the kinetics of toxin formation by *Fusarium graminearum.* Miller et al. [1983] found a rapid increase of DON concentration in corn cobs inoculated in the field with *Fusarium graminearum*, whereas the production of ZEA increased at a significantly lower rate, after a delayed onset. If this time course of toxin formation is to be observed also in wheat this may contribute to the higher content of DON compared to ZEA at the time of harvest.

The low frequency and levels of 3- and 15-ADON may have resulted from the partial deacetylation of both toxins into DON in the field. This transformation may have been due to the activity of the DON producing *Fusarium* mycelium, of bacteria and yeasts occurring on wheat plants and/or of the host plant enzymes. Likewise, the almost complete absence of detectable α- and β-ZOL from wheat may result at least in part from the conversion in the field of these toxins into ZEA (for literature see Müller and Schwadorf, 1993).

Regarding the differences between years, it is interesting to note that the frequency of DON was high throughout the whole survey whereas its mean level was much higher in samples from 1987 compared to those from the other years (Table II). However, both the incidence and mean level of ZEA was higher in the 1987 samples compared to the other years (Table II). The explanation for these findings may be due to a combined effect of precipitation and of the aforementioned kinetics of the formation of the two toxins.

Due to the early onset of DON production its concentration may rise significantly before the time of harvest even under dry summer conditions, this leading to a consistently high incidence. However, in a wet summer, the mycelium is able to form higher amounts of this toxin due to the longer period before the decrease of kernel moisture content arrests its production. In contrast, due to the delayed onset of ZEA production it is produced in a low percentage of kernels in a dry summer and levels at harvest are simply low. However, the slower decrease of kernel moisture content in a wet summer may enable the mycelium to produce detectable ZEA concentrations in a higher percentage of kernels than in a dry summer, and may result in significantly higher mean and maximum concentrations at harvest as well. It remains to be explained why in 1993 the incidence of ZEA was markedly higher than in 1989–1992 though the mean precipitation in the summer months of this year was only slightly above that in 1992 (Table I). The data reviewed by Gareis et al. [1989] suggest an overall incidence of ZEA in wheat of European origin of 25%. However, there was a wide range of incidences and levels described by different authors. The present study suggests that this resulted from different climatic conditions.

As can be seen from Table II, the incidence of 3-ADON decreased from 1987 through 1992 whereas that of NIV increased during this time. This may have resulted from a decreased frequency of *Fusarium* strains producing 3-ADON, accompanied by an increased frequency of NIV producing *Fusarium* strains, with both effects resulting from the dry climate during 1989–1992. However, it remains to be explained why the incidence of 3-ADON (along with its mean content) increased markedly in 1993, accompanied by a decreased incidence of NIV.

Based on the maximum limits suggested in Canada the risk for ruminants and poultry posed by DON and ZEA in wheat harvested in the area surveyed was rather low in 1989–1993. It was somewhat higher in 1987, i.e., in the year with high precipitation. Although this risk evaluation is useful many questions remain open. For example, only little information is available in the literature on the effects in livestock of wheat and other grains contaminated with more than one *Fusarium* toxin. However, this co-occurrence occurs rather frequently. One of the combinations observed by Müller and Schwadorf [1993] in wheat was that of DON and NIV, but the co-existence of DON with type-A trichothecenes has also been found. It is well known that NIV, T-2, HT-2, and DAS are more acutely toxic in laboratory animals than DON [Wannemacher et al., 1991]. Therefore, for evaluating the risks posed to livestock the whole spectrum of *Fusarium* toxins as well as their interactions (additive or synergistic) should be considered.

The findings of the present study stress the need for regular screening cereals for *Fusarium* toxins, particularly in years with high precipitation in the summer months.

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